Selection for Establishment Capacity in Reed Canarygrass

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ABSTRACT

Establishment of reed canarygrass (*Phalaris arundinacea* L.) is seriously impaired by relatively low seeding vigor and growth rate. Poor soil–seed contact, intense competition from annual weeds, and infrequent clipping during the establishment year exacerbate this problem. The objectives of this study were (i) to select reed canarygrass populations for establishment capacity in the presence of annual weeds, (ii) to evaluate progeny for progress from selection, and (iii) to determine mechanisms for improved establishment potential in selected populations. Two cycles of selection for survival under clipping and weed competition were completed, the first involving selection among spaced plants and the second involving selection within seeded plots. Selection for increased establishment capacity increased ground cover in October of the seeding year by 29% and tiller density in May of the following year by 36%, averaged over five cultivars. Seed mass consistently decreased with selection for increased establishment capacity, but emergence rate increased in all cultivars by an average of 18.1%. Three of the five cultivars (Palaton, Vantage, and Venture) responded to selection with increased shoot and/or root fresh mass, with shoot-mass responses generally larger than root-mass responses. Selected populations of Vantage and Venture also had small decreases in the time required for tiller initiation. Bellevue and Rival showed no responses of seedling traits to selection. Seedling fresh mass was the most important factor regulating genetic variability for establishment capacity, but there was some variation in the mechanism of improved establishment capacity among populations.

REED CANARYGRASS is an undomesticated forage crop. Cultivated germplasm is only one or two cycles of selection and sexual recombination removed from wild germplasm. Seed shattering, seed dormancy, and low seedling vigor are characteristics of nearly all reed canarygrass germplasm. Low seedling vigor, resulting in slow and/or poor establishment, is the most important factor limiting agricultural use of reed canarygrass. Reed canarygrass seedlings must survive shading, competition, and perhaps allelopathic effects from annual weeds before they become autotrophic and are capable of creating an established sward. Increased seeding rates have little effect on short-term establishment of reed canarygrass under competitive conditions (Casler et al., 1999).

Various strategies have been used to improve establishment potential of forage crops. Seed size is a heritable trait that has responded to selection and is often positively correlated with seedling vigor (Berdahl and Barker, 1984; DeHaan et al., 2001; Trupp and Carlson,

1971; Twamley, 1974). However, selection for larger seeds may be insufficient as a sole selection criterion to improve seedling vigor (McLean and Nowak, 1997). Seedling vigor and/or germination rate have responded to selection in several species (DeHaan et al., 2001; Lawrence, 1977; Townsend, 1974; Voigt and Brown, 1969).

If seeds are placed in a stressful environment, selection pressure can be simultaneously applied for seedling vigor and stress tolerance. Selection for emergence from deep planting has improved establishment capacity for field plantings under dryland conditions (Berdahl and Barker, 1984; Lawrence, 1977). Selection for germination and seedling vigor at suboptimal temperatures resulted in faster seedling growth under field conditions (Klos and Brummer, 2000a, 2000b). Because reed canarygrass must survive and grow in an environment dominated by fast-growing annuals, seed size or seedling vigor under competitive conditions may be the most important traits for improving establishment.

Among the perennial grasses, establishment rate is inversely proportional to perenniality (Casler et al., 1999; Undersander et al., 2001). A tradeoff most likely occurs between shoot growth (seedling vigor) and root growth, a structural prerequisite for perenniality. Long-term perennial grasses, such as reed canarygrass, appear to devote resources early in their life cycle to root and rhizome development at the expense of seedling vigor and shoot growth (Undersander et al., 2001). Conversely, perennial grasses with superior seedling vigor tend to be short-term perennials, less capable of long-term survival under competitive or stressful conditions (Undersander et al., 2001).

The objectives of this study were (i) to select reed canarygrass plants and populations for establishment capacity in the presence of annual weeds, (ii) to evaluate progeny for progress from selection, and (iii) to determine mechanisms for improved establishment potential in selected populations.

MATERIALS AND METHODS

Experiment 1: Initial Selections

This study was initiated with certified seed of four reed canarygrass cultivars: Palaton, Rival, Vantage, and Venture. Four hundred fifty seedlings of each cultivar were established in 2.5 by 2.5 by 5 cm pots in the glasshouse in February 1993. Seedlings, consisting of four to six tillers, were transplanted to a Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll) soil in May 1993 at Arlington, WI. Seedlings were irrigated once to aid their short-term establishment. Seedlings were arranged in a randomized complete block design with 15 replicates and plots that consisted of a row of 30 seedlings. All seedlings were spaced on perpendicular 0.9-m centers.

During the remainder of 1993 and all of 1994, weeds were controlled only by clipping. The weed population consisted primarily of the annuals crabgrass [*Digitaria sanguinalis* (L.)

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Scop.], green foxtail [*Setaria viridis* (L.) Beauv.], barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], fall panic [*Panicum dichotomiflorum* Michx.], lambsquarter (*Chenopodium album* L.), and redroot pigweed (*Amaranthus retroflexus* L.), with each species uniformly distributed across the experimental area to form a uniform and dense ground cover. The entire experiment was clipped to a stubble height of 9 cm whenever the weed canopy reached an average height of approximately 20 cm. The experiment was clipped six times in 1993 and seven times in 1994. Fertilizer was not applied to this experiment. Clipping was accomplished with a flail chopper to remove all clipped forage. Soil tests revealed adequate levels of P and K.

Maximum plant diameter of each plant's crown was measured in early spring 1994 and 1995. Percentage mortality of each plot was determined from the plant diameter data. Data were analyzed by analysis of variance, assuming cultivars to be fixed and blocks to be random.

Forty-five plants were selected for high plant diameter in spring 1994 and 1995 combined with high spring vigor in May 1995. Plant diameter was used as a selection criterion because it measured a plant's ability to compete with weeds for soil surface area. Spring vigor (visual evaluation of plant canopy height when most plants were approximately 15 to 20 cm tall) was used as an indicator of stored carbohydrate reserves for rapid spring growth. These 45 plants were planted to a polycross block in May 1995, arranged in a randomized complete block design with four replicates. Plants were fertilized with 56 kg N ha^{-1} in early spring 1996 and 1997 and polycross seed was produced in July of 1996 and 1997. Seed was bulked in equal quantities for each polycross family produced in each year. This population of 45 polycross families was named WR97.

Also in May 1995, 500 plants each of five reed canarygrass cultivars (Bellevue, Palaton, Rival, Vantage, and Venture) were transplanted into isolated crossing blocks at Arlington, one block for each cultivar. Ten-week-old seedlings were transplanted by hand into rows that were 0.9 m apart with a 0.3-m spacing within rows. Each block was isolated by a minimum of 100 m from other reed canarygrass. Weeds were controlled by pre-emergence herbicide applications and hand weeding. Plants were fertilized with 56 kg N ha^{-1} in early spring 1996 and 1999 and seed was harvested in July of 1996 and 1999. Seeds were bulked in equal quantities for each plant within each block. Seed harvested from these five crossing blocks was used to represent the original cultivars in all remaining studies.

Experiment 2: Cycle 1 of Within-Family Selection

Seed of each of the 45 polycross families was tested for germination according to the Association of Official Seed Analysts standards (AOSA, 1998). Germination was computed separately for each of two 0.25-g seed samples per family. Seeding rates for each family were adjusted for germination percentage and seed mass to a constant number of pure live seeds (PLS) per unit area.

The 45 polycross families and the five cultivars (represented by seed from the 1996 Arlington seed increases) were planted in four four-replicate randomized complete block experiments in April 1998 at Arlington, WI. The four experiments consisted of three seeding rates without companion crop (64, 128, and 256 PLS m^{-2}) and one seeding rate (256 PLS m^{-2}) with a red clover (*Trifolium pratense* L.) companion. The three seeding rates were equivalent, on average, to seeding rates of 0.6, 1.2, and 2.4 kg ha^{-1} (approximately 5, 10, and 20% of the normal recommended rate). The red clover was planted at a rate of 7 kg ha^{-1} with a cultipacker seeder over the top of the seeded reed canarygrass plots. Plots were 0.9 by 1.4 m and consisted of five drilled rows, spaced 15 cm apart. Each of the five cultivars

was seeded to nine plots per replicate to improve statistical precision of the comparison between cultivars and polycross families. This and all other field experiments followed a previous year crop of soybean [*Glycine max* (L.) Merr.] and the seedbed was prepared by chisel plowing and cultimulching.

Management was similar to that for Experiment 1—weeds were controlled only by clipping when the weed canopy reached a height of approximately 20 cm. The weed population was relatively uniform, largely consisting of the species described above. The experiment was clipped three times in 1998. Fertilizer was not applied to this experiment. Soil tests revealed adequate levels of P and K.

In late April 1999, tiller density of each plot was determined by counting all emerged reed canarygrass tillers within two 0.1-m^2 sampling frames placed at random positions within each plot. Maximum reed canarygrass tiller height was 15 cm. There were no weeds to interfere with tiller counts. Red clover was killed with an early spring application of 0.45 kg a.i. ha^{-1} 2,4-D [(2,4-dichlorophenoxy) acetic acid]. Tiller density data were analyzed by analysis of variance assuming all effects to be random. Nearest neighbor adjustment was used to adjust family and cultivar means for spatial variation (Casler, 1999). Comparisons between cultivars and families were made using contrasts. Cultivar means were compared using the protected LSD (Carmer and Walker, 1982). Heritability of tiller density, on a half-sib family mean basis, was computed as $h^2 = s^2_F/(s^2_F + s^2_e/r)$, where s^2_F = the family variance component, s^2_e = the error variance component, and r = number of replicates.

Experiment 3: Selection Evaluation

Immediately after tiller counts were made, Experiment 2 was fertilized with 56 kg N ha^{-1} and left undisturbed until the initiation of seed shattering. The experiment had been treated with 1.12 kg ha^{-1} alachlor [2-chloro-*N*-92,6-diethylphenyl)-*N*-(methoxymethyl)-acetamide] and 0.07 kg ha^{-1} imazethapyr {(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} for weed control. Seed was harvested individually from every plot in Experiment 2. All seeds harvested from the 45 polycross families and the five check cultivars in Experiment 2 were separately bulked for each family and cultivar. These seeds represented the culmination of one cycle of natural selection within cultivars and families for establishment capacity. For the check cultivars, this represented a single cycle of selection. For the polycross families, this seed represented a second cycle of selection, albeit with a different protocol and marginally different selection criterion than that of the first cycle (Experiment 1). This selection method can be described as phenotypic selection within maternal lines, without pollen control. Seed of the 45 polycross families was bulked in equal quantities and named WR99.

The 10 polycross families with the highest establishment capacity, measured by tiller density, were identified from Experiment 2 and labeled within the original 45-clone polycross block from Experiment 1. The polycross block was fertilized with 56 kg N ha^{-1} in early May 1999. Shortly after heading, the remaining 35 clones within this polycross block were mowed to prevent pollination and seed set. Seed was harvested on four replicates of the selected 10 clones, threshed, cleaned, and bulked in equal quantities for the 10 clones. This population was named WR00.

Experiment 3a: Establishment Capacity

The five cultivars (represented by seed from the 1999 Arlington seed increases), their five cycle-1 progeny populations

from Experiment 2 (seed produced in 1999), and three experimental populations (WR97, WR99, and WR00) were seeded in April 2001. The experimental design was a randomized complete block with eight replicates at each of four locations. The locations were: Arlington (Plano silt loam; 43°20′N, 89°23′W), Ashland (Ontonagon silty clay loam [very-fine, mixed, active, frigid Haplic Glossudalf]; 46°35′N, 90°54′W), Lancaster (Fayette silt loam [fine-silty, mixed, superactive, mesic Typic Hapludalf]; 42°50′N, 90°47′W), and Marshfield (Withee silt loam [fine-loamy, mixed, superactive, frigid Aquic Glossudalf]; 44°39′N, 90°08′W). Seeds were tested for germination (AOSA, 1998) and the seeding rate was 64 PLS m^{-2}. Plot size was 0.9 by 1.5 m.

Plots were clipped two or three times during 2001 to control annual weeds. Ground cover of reed canarygrass was determined in October 2001 using a 0.75 by 0.75 m grid divided into 25 0.15 by 0.15 m squares, as described by Vogel and Masters (2001). Tiller density was determined in May 2002 using the same grid. Reed canarygrass tillers were counted in four random 0.15 by 0.15 m squares per plot. Establishment potential was defined as the degree to which reed canarygrass covered the ground and tiller density. Data were analyzed by analysis of variance assuming all effects to be random, except populations, which were fixed. Comparisons between cultivars and selected populations were made using contrasts. Population WR99 was compared to population WR97 as a measure of the effect of one cycle of within-family selection for establishment capacity. Population WR00 was compared to population WR97 as a measure of the effect of one cycle of among-family selection for establishment capacity.

Experiment 3b: Forage Yield

These 13 populations were also seeded at Arlington and Marshfield using a normal seeding rate of 1280 PLS m^{-2}, which was approximately equivalent to 11.2 kg ha^{-1}. The experimental design and plot size were identical to that used for Experiment 3a, except for the use of 16 replicates instead of eight. Plots were clipped two or three times during 2001 to control annual weeds. Plots were harvested three times each in 2002 through 2004, in early June, late July, and late October at a 9-cm cutting height. Forage yield was determined for each plot, but dry matter was assumed to be constant across plots and was based on a random dry matter sample. Total forage yield was analyzed by analysis of variance as described for Experiment 2a.

Experiment 4: Establishment Mechanisms

The following traits were hypothesized to have a potential positive effect on improving establishment of reed canarygrass seedings: seed mass, rate of germination, seedling height, shoot fresh and dry mass, root fresh and dry mass, rate of tillering, and tiller number. Three experiments were designed to measure these traits on the 13 populations described above and to determine associations of these traits with establishment capacity. Seed lots used for these experiments were identical to those used for Experiments 3a and 3b.

Experiment 4a: Seed Mass and Emergence

Ten 100-seed lots were counted from each of the 13 populations evaluated in Experiments 3a and 3b. Seed mass was determined for each seed lot, after which they were planted in sand flats in the greenhouse. Flats were filled with sand to a depth of 4 cm and seeds were planted at a 5-mm depth. Each 100-seed lot was planted in a 0.5-m row and adjacent rows were 5 cm apart. Flats were watered and covered with clear plastic covers until the first seedling emerged on the seventh day after planting, after which the covers were removed. The number of emerged seedlings per row was counted daily from 7 to 21 d after planting. The experiment was designed as a randomized complete block with five replicates in each of two runs (January and February 2004). A 16-hour photoperiod with a minimum of 400 μmol m^{-2} s^{-1} of photosynthetically active radiation was maintained using high-pressure sodium lamps.

Based on the shape of emergence curves as a function of time, emergence was modeled using the log-linear regression model: $Y_i = a + b[\ln(X_i)]$, where Y_i = emergence on the ith day (X_i). The expected number of days to 50% emergence (D50) was computed as D50 = $\exp\{[(E/2) - a]/b\}$, where E = emergence on day 21. Seed mass, log-linear slope (b), and D50 for each experimental unit were analyzed by analysis of variance assuming all effects to be random, except populations, which were fixed. Comparisons between cultivars and selected populations were made using contrasts.

Experiment 4b: Root and Shoot Vigor

Twelve 100-PLS lots were counted from each of the 13 populations using emergence on day 21 from Experiment 4a as an estimate of PLS for each seed lot. Flats were filled with a 50:50 (v:v) sand/silt loam soil mixture to a depth of 4 cm and seeds were planted at a 5-mm depth. Each 100-PLS lot was planted in a 0.5-m row and adjacent rows were 5 cm apart. Flats were watered daily. The experiment was designed as a split-plot in a randomized complete block with two replicates in each of two runs (March and April 2004)—harvest dates were whole plots and populations were subplots. Harvest dates were 16, 23, and 30 d post-emergence, corresponding approximately to the two-leaf, three-leaf, and four-leaf morphological stages.

At harvest, the height from soil level to the longest fully extended leaf blade within each row was measured within each row. Shoots were clipped at soil level, washed free of sand and soil, blotted dry with paper towels, and weighed. Roots were removed and washed free of sand and soil using a 5-mm screen and a high-pressure water jet, blotted dry with paper towels, and weighed. Shoots and roots were dried at 60°C for 3 d then weighed again to determine dry mass for each experimental unit. The slopes of the linear regressions of shoot and root fresh and dry mass on number of days post-emergence were computed for each replicate and run. All variables were analyzed separately for each harvest date using analysis of variance and assuming all effects to be random, except populations, which were fixed. Comparisons between cultivars and selected populations were made using contrasts.

Experiment 4c: Tillering

The 13 populations were planted into 5-cm-square containers that were 5 cm deep. Each container was planted with two to four seeds in late November 2004, which were thinned immediately after emergence to one seedling per cell. Emergence date of each seedling was recorded. An experimental unit consisted of five cells which were filled with a 50:50 (v:v) peat moss/silt loam soil mixture. The experiment was designed as a randomized complete block with 12 replicates (60 total seedlings per population). A 16-hour photoperiod with a minimum of 400 μmol m^{-2} s^{-1} of photosynthetically active radiation was maintained using high-pressure sodium lamps.

The date of emergence of the first new tiller was recorded for each seedling. At 35 d post-emergence, plants were clipped to a 7-cm stubble height and fertilized with the equivalent of 80 kg N ha^{-1}. The number of tillers was counted on each

seedling 42 d post-emergence. Days to tiller initiation and tiller number were analyzed using analysis of variance and assuming all effects to be random, except populations, which were fixed. Comparisons between cultivars and selected populations were made using contrasts.

Synthesis

All variables with significant differences among the 13 population means ($P < 0.01$) were entered into a multiple regression analysis to explain variation in ground cover or tiller density of Experiment 3a. Backward stepwise elimination with $P = 0.01$ was used to eliminate variables from the regression model.

RESULTS AND DISCUSSION
Experiment 1: Initial Selections

Reed canarygrass cultivars differed in crown diameter at both measurement dates and in plant mortality one year after transplanting (Table 1). Crown diameter increased three-fold during the second year, despite the presence of annual weeds. The increase in crown diameter was relatively constant across cultivars. Crown diameter and plant mortality, both measures of competitive ability against weed populations were negatively correlated with each other, ranking the cultivars in nearly the opposite order. The 45 plants selected from this experiment were distributed among the cultivars as follows: 12 from Palaton, 9 from Rival, 4 from Vantage, and 20 from Venture. Vantage had the highest mean crown diameter and lowest mean plant mortality, but the lowest spring vigor The large number of selections made from Venture were a result of its high phenotypic variance for crown diameter and relatively high spring vigor in May 1995.

Experiment 2: Cycle 1 of Within-Family Selection (Initial Selection Evaluation)

Tiller density increased by 0.69 ± 0.20 ($R^2 = 0.92$) for each additional seed planted (Table 2). The slope less than unity reflected a diminishing return from increased seeding rates even at these extremely low seeding rates. This led to a decrease of -0.60 ± 0.07 ($R^2 = 0.99$) tillers per 100 seeds planted as seeding rate was increased. Reed canarygrass tiller density in mixture with red clover was 41% of that at the same seeding rate in the annual weed environment, resulting in 0.45 tillers per seed planted. These extremely low seeding rates were

Table 1. Mean crown diameter and mortality of reed canarygrass spaced plants transplanted to the field in May 1993. Means are over 15 replicates and, for crown diameter, up to 30 plants per plot (Experiment 1).

| Cultivar | Variable and date | | |
	Crown diameter May 1994	Plant mortality May 1994	Crown diameter May 1995
	cm	%	cm
Palaton	12	1.3	39
Rival	10	4.7	32
Vantage	16	0.4	45
Venture	13	1.1	37
LSD(0.05)	2	1.8	4

Table 2. Mean tiller density of reed canarygrass cultivars and polycross families in April 1999, one year after seeding, determined at three pure-stand (P) seeding rates and one seeding rate overseeded with red clover (RC). Means are over four replicates and stated number of families (Experiment 2).

| Cultivar or group | Establishment treatment: Companion crop and seeding rate (PLS m^{-2}) | | | |
	P-64	P-128	P-256	RC-256
	tillers m^{-2}			
Bellevue	102b†	186c	257b	83b
Palaton	150a	218b	258b	98b
Rival	72c	153d	185d	75b
Vantage	166a	256a	291a	133a
Venture	101b	164d	220c	81b
High family	238	331	439	343
Low family	118	183	227	89
Palaton families (12)	174j	266j	335j	125j
Rival families (9)	157j	252j	324j	151j
Vantage families (4)	198i	292i	406i	233i
Venture families (20)	190i	264j	325j	126j
Mean of all families	180m	265m	335m	140m
Mean of all cultivars	122n	197n	239n	97n
Overall mean	149	230	288	117

† Means within groups and columns followed by different letters are significantly different at the 0.05 level (a–d and i–j, determined by protected LSD) or the 0.01 level (m–n, determined by contrasts).

intended to simulate situations that characterize typical establishment conditions for reed canarygrass in farming systems. Relatively poor seedbed preparation, poor soil–seed contact, and intense competition from annual weeds or existing pasture species results in a very small number of viable seedlings on working farms. Establishment at these low seeding rates indicated that reed canarygrass can become established even if very few seeds are capable of germination, emergence, and competition against existing vegetation. The high tillering capacity and rhizomatous nature of this species can partly overcome deficiencies in the number of seeds that are capable of producing established plants. Reed canarygrass is extremely slow to establish relative to other grasses (Casler et al., 1999), but rhizome and tiller production can make up for this deficiency over time (Undersander et al., 2001).

Vantage had the highest ranked tiller density 1 yr after planting at all seeding rates and with the red clover companion, differences that were significant at all but the lowest seeding rate (Table 2). Rival consistently had the lowest tiller density, which was significantly lower than all other cultivars for two of the four plantings. Regardless of whether establishment capacity was measured as crown diameter or mortality of spaced plants (Experiment 1) or tiller density of seeded plots at various seeding rates or with a red clover competitor (Experiment 2), cultivar rankings were similar. This result indicated that each of these variables provided an adequate relative estimate of the potential for a reed canarygrass cultivar to establish itself under competition and that competition from annual weeds or red clover had a similar relative effect on the five cultivars.

Polycross families were highly variable in tiller density at all seeding rates and for the mixture with red clover. Narrow-sense heritability for tiller density was moderate for all establishment treatments, ranging from 0.45 to 0.51. The phenotypic correlation was positive for poly-

cross family means across the four establishment treatments, ranging from 0.34 to 0.61, indicating a general continuity in phenotypic variability across the four seeding rates and companions.

The initial selection of spaced plants with high crown diameter and spring vigor following 2 yr of frequent mowing under annual weed competition appears to have been successful in increasing establishment capacity (Table 2). The mean of all selected polycross families exceeded the mean of the cultivars by 34 to 47% across the four establishment treatments ($P < 0.01$). Furthermore, the mean of the polycross families was numerically higher than the mean of Vantage for each establishment treatment. Polycross families derived from Vantage were highest in tiller density at all four establishment treatments, while those from Rival were lowest in tiller density for three of four treatments. This general trend indicated a positive correlation between parental performance in the original spaced-plant nursery and progeny performance under the four establishment treatments, confirming that tiller density is a heritable trait and that it has a positive genetic correlation with crown diameter and plant survivorship.

Experiments 3a and 3b: Selection Evaluation

The initial cycle of selection for establishment capacity, based on low seeding rates, numerically increased both ground cover in October of the establishment year and tiller density in May of the following year for all five cultivars (Table 3). This numerical increase in ground cover or tiller density was significant for nine of the 10 comparisons made for the five cultivars. Averaged over all five cultivars, this represented a 28% increase in ground cover (23.1 vs. 18.0%) and a 36% increase in tiller density (93.8 vs. 68.8 tillers m^{-2}) from a single cycle of selection within cultivars ($P < 0.01$). These results confirm those from the first two experiments, indicating that establishment capacity is a heritable trait in reed canarygrass, there is a tremendous amount of genetic variability for this trait, and it is relatively easy to make progress.

The three populations WR97, WR99, and WR00 represent increasingly intensive selection efforts to produce an improved population from the 45 polycross families selected from Experiment 1. WR97 represented the original 45 families, while WR99 represented the

Table 3. Mean ground cover in October of the establishment year and tiller density in May of the following year for seven reed canarygrass cultivars or populations and their progeny selected for establishment capacity. Means are over eight replicates and four locations (Experiment 3a).

Cultivar or population	Ground cover			Tiller density		
	Original	Selected	P value	Original	Selected	P value
		%			Tillers m^{-2}	
Bellevue	20	25	<0.01	77	100	<0.01
Palaton	15	22	<0.01	62	78	0.04
Rival	22	25	0.06	87	108	0.01
Vantage	20	23	0.11	79	95	0.04
Venture	13	20	<0.01	38	89	<0.01
WR99	17	21	0.08	68	82	0.06
WR00	17	31	<0.01	68	111	<0.01
LSD(0.05)	5			21		

Table 4. Mean forage yield, summed over three harvests, for seven reed canarygrass cultivars or populations and their progeny selected for establishment capacity. Means are over eight replicates at Arlington and Marshfield, WI in 2002 through 2004 (Experiment 3b).

Cultivar or population	Original	Selected	P value
		Mg ha^{-1}	
Bellevue	9.70	10.23	0.02
Palaton	9.40	9.80	0.08
Rival	10.03	9.92	0.62
Vantage	10.03	10.31	0.20
Venture	9.50	9.65	0.49
WR99	10.03	10.05	0.96
WR00	10.03	10.19	0.55
LSD(0.05)	0.43		

completion of the first cycle of within-family selection for establishment from seed, and WR00 represented a single cycle of among-family selection with a selection intensity of p = 0.22. Their mean ground cover and tiller density increased directly in response to increasing intensity of selection, with WR00, the 10-clone synthetic having the highest mean in the experiment for both variables (Table 3). These results further support the heritable nature of establishment capacity in this species. Furthermore, these results were consistent across the four locations, showing no evidence of genotype × location interaction. This suggests that the inheritance of establishment capacity in reed canarygrass is relatively simple, largely a function of additive effects that are relatively insensitive to environmental effects. Thus, it is likely that selection has acted upon a relatively small number of loci controlling this trait.

As a result of selection for increased establishment capacity, forage yield increased in one of five cultivars, but was not significantly changed in the remaining cultivars or in WR99 or WR00 (Table 4). Despite this lack of significance in the individual comparisons, probably due to insufficient replication, the general trend was for an increase in forage yield, which averaged 2.6% (9.98 vs. 9.73; $P = 0.01$) across the five cultivars. Because of the high seeding rates for this study, all plots established well during the seeding year. Furthermore, the difference between selections and cultivars was consistent across locations and years, showing statistical significance ($P < 0.05$) for five of six location–year combinations. Thus, the correlated responses in forage yield were not a result of short-term establishment effects, but appeared to represent improvements in forage yield potential. Selection for increased seed size or speed of germination did not affect forage yield of two other perennial grasses (Lawrence, 1977; Jessen and Carlson, 1985).

Experiments 4a, 4b, and 4c: Establishment Mechanisms

Surprisingly, seed mass decreased as a result of selection for establishment capacity in four cultivars (numerically in all five cultivars), averaging 4.4 to 14.2% reduction (data not shown). Averaged across the five cultivars, seed mass decreased by 9.0% (85 to 77 mg 100 seeds^{-1}: $P < 0.01$). Responses were similarly negative, but of a much higher magnitude for the two

composite populations, with decreases of 10.4% for WR99 and 41.6% for WR00. These results are puzzling because seed mass is generally positively associated with establishment capacity, particularly under competitive conditions (Venable and Brown, 1988). Selection for increased seed mass results in increased seedling vigor (Trupp and Carlson, 1971; Twamley, 1967) and vice versa (Twamley, 1974). There are three possible explanations for our observations.

First, owing largely to their consistency across selection events, these observations may indicate an alternative strategy to large seeds for enhancing seedling vigor and the probability of seedling establishment. Perennial grass seedlings undergo a phase change, transitioning from a heterotrophic phase, in which they are dependent on seed reserves, to an autotropic phase when they become dependent on photosynthesis from leaves and water uptake from roots (Whalley et al., 1966). If small seeds make this transition faster than large seeds, their seedlings may have a fitness advantage over seedlings from larger seeds. Such a fitness advantage might be manifested in more rapid root and/or shoot development, regulated by earlier initiation of adventitious roots and/or new shoots. Phase changes in plants are genetically regulated (Poethig, 2003) and genetic variability for timing of phase changes have been observed in several species (Jordan et al., 1999; Scott et al., 1999; Vega et al., 2002).

Second, because seed mass is highly sensitive to environmental effects (Boe, 2003), these may be environmental effects. For the five cultivars, seeds of the original cultivars were produced from spaced plants, while seeds of the selected populations were produced in situ from Experiment 2, conditions which resulted in sparse swards with 149 to 288 tillers m^{-2} (Table 2). Differential plant spacing may have an environmental effect on seed mass. For the composite populations, seed was produced in three different years (1997, 1999, and 2000). If seed mass of reed canarygrass is positively related to seedling vigor and establishment capacity, as in numerous other species (Kitchen and Monsen, 1994; Smart and Moser, 1999), reductions in seed mass for all selected lines, due to environmental effects, would result in a downward bias to any estimates of genetic gain from selection. Because all experiments were planted with a constant number of pure live seeds across all populations, differences between selected and unselected lines for other traits may be considered to represent genetic gains due to selection. The potential downward bias to these effects, from possible environmental effects of reduced seed mass, should be recognized.

Third, they may be a result of drift during the selection process. Drift could have a reasonably uniform effect across selected populations and may not affect all traits evaluated on these populations, depending on their sensitivity to inbreeding depression (Falconer, 1989). In switchgrass (*Panicum virgatum* L.), seed size had no effect on establishment capacity once a seedling had two or more adventitious roots (Smart and Moser, 1999). Thus, an effect of variation in seed mass may not be observed after a critical stage of seedling develop-

ment, potentially including a phase change from heterotropic to autotrophic.

Rate of seedling emergence was consistently higher in all selected populations compared to their respective parent population, although none of these differences were significant due to insufficient replication (data not shown). Averaged across the five cultivars, this effect was significant, averaging an 18.1% increase in seeding emergence rate (26 vs. 22 seedlings ln[d]$^{-1}$; $P = 0.05$). Thus, it appears that an increased rate of seedling emergence may be a factor in the increased establishment rate of the selected populations. Similarly, selection for increased germination rate increased field emergence and seedling height in alfalfa, *Medicago sativa* L. (Klos and Brummer, 2000b). Variation among the 13 population means was not significant for the number of days to 50% emergence (D50).

Seedling height did not differ among the 13 populations for any of the three harvest dates and was excluded from all further analyses. Fresh and dry mass were highly correlated with each other for both shoots and roots ($r = 0.91$ to 0.99: $P < 0.01$), so all conclusions were identical based on analyses of either fresh mass or dry mass. Fresh mass of both shoots and roots were chosen for presentation and use in all further analyses.

At 16 d post-emergence, Palaton and Venture both showed large and fairly consistent increases in shoot and root fresh mass (data not shown). Responses were greater for Palaton than for Venture and shoot mass responded more to selection than root mass. Both composite populations responded similarly to selection, showing increases in shoot and root fresh mass; responses were greatest for shoot mass. At 23 d post-emergence, selection responses for shoot and root fresh mass were significant for Palaton, Vantage, and Venture (data not shown). These responses were consistently high, ranging from 37 to 63% increase in fresh mass. Selection responses at 23 d were inconsistent and nonsignificant for the two composite populations.

At 30 d post-emergence, selection responses for shoot fresh mass were still significant for Palaton, Vantage, and Venture, but selection responses for root fresh mass were significant only for Venture (Table 5). Shoot fresh mass at 30 d was increased in both WR99 and WR00, but neither population showed any changes in root fresh mass at 30 d.

Table 5. Mean shoot and root fresh mass for seven reed canarygrass cultivars or populations and their progeny selected for establishment capacity. Means are over four replicates 30 d after emergence (Experiment 4b).

Cultivar or population	Shoot fresh mass			Root fresh mass		
	Original	Selected	P value	Original	Selected	P value
		g			g	
Bellevue	26.2	30.7	0.14	15.6	16.3	0.79
Palaton	18.5	29.3	<0.01	10.5	10.4	0.99
Rival	29.4	29.0	0.91	12.6	14.6	0.46
Vantage	24.3	31.4	0.02	8.5	10.6	0.44
Venture	17.0	27.5	<0.01	7.5	13.1	0.04
WR99	25.9	31.7	0.06	11.4	10.5	0.73
WR00	25.9	36.1	<0.01	11.4	11.8	0.88
LSD(0.05)	6.1			5.4		

The phenotypic correlation coefficient between shoot fresh mass and root fresh mass decreased with seedling age, from $r = 0.96$ ($P < 0.01$) at 16 d, to $r = 0.84$ ($P < 0.01$) at 23 d, and $r = 0.43$ ($P = 0.14$) at 30 d. Thus, shoot and root fresh mass were closely coupled in young seedlings, but their relationship declined as seedlings aged. This decoupling was responsible for changes in the relative importance of shoot vs. root fresh mass responses to selection, with root fresh mass decreasing in its response to selection over time in Palaton and Vantage (Table 5). Shoot and root mass of Kura clover (*Trifolium ambiguum* M. Bieb.) were also positively correlated with each other, generally resulting in positive correlated responses of both traits to selection for increased seedling vigor (DeHaan et al., 2001).

Shoot and root mass both increased linearly with time between 16 and 30 d post-emergence (Fig. 1). The 13 populations differed for linear regression coefficients of both shoot and root mass on post-emergence time. Linear responses for shoot mass were numerically increased in four of the five cultivars, but because of insufficient replication, these differences were not significant (Table 6). Averaged over cultivars, selected populations had a 32.3% greater linear response to post-emergence time than the original cultivars (1.48 vs. 1.12 g d^{-1}; $P < 0.01$; Fig. 1). Differences between cultivars and selected populations in linear response of root mass to post-emergence time followed a similar pattern (Fig. 1), but
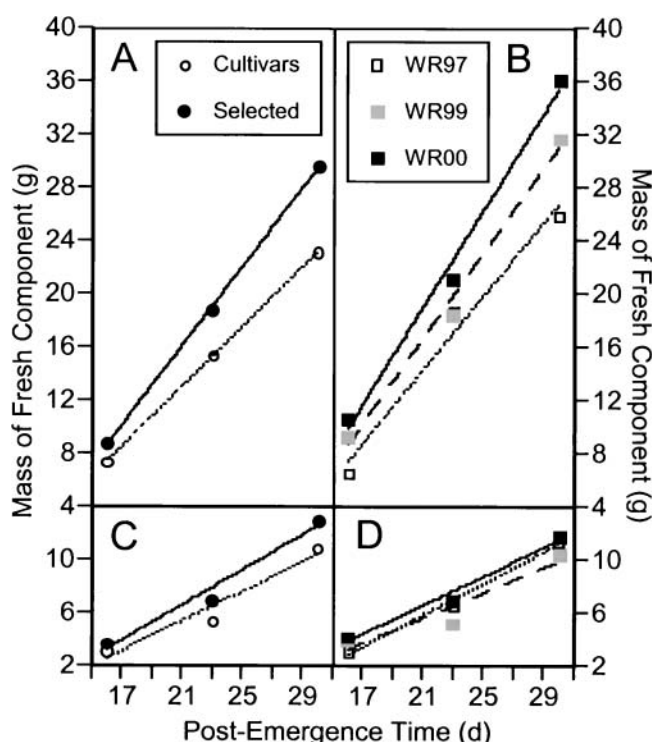

Fig. 1. Linear regressions of shoot and root mass on post-emergence time for reed canarygrass cultivars and various populations selected for improved establishment capacity: (A) shoot mass of five cultivars and five selected populations, (B) shoot mass of three composite populations representing increasing selection intensities (WR00 > WR99 > WR97), (C) root mass of five cultivars and five selected populations, and (D) root mass of three composite populations.

Table 6. Mean linear regression coefficients for shoot and root fresh mass as a function of post-emergence time for seven reed canarygrass cultivars or populations and their progeny selected for establishment capacity (Experiment 4b).

Cultivar or population	Shoot fresh mass			Root fresh mass		
	Original	Selected	*P* value	Original	Selected	*P* value
	g d^{-1}			g d^{-1}		
Bellevue	1.14	1.60	0.15	0.84	0.93	0.69
Palaton	1.02	1.43	0.20	0.61	0.49	0.56
Rival	1.43	1.35	0.81	0.61	0.74	0.51
Vantage	1.13	1.62	0.13	0.37	0.48	0.60
Venture	0.88	1.42	0.09	0.38	0.69	0.14
WR99	1.39	1.60	0.51	0.60	0.47	0.55
WR00	1.39	1.82	0.18	0.60	0.55	0.81
LSD(0.05)	0.64			0.41		

these differences were too small to be statistically significant (Table 6). There was a progressive increase in linear response of shoot mass for the three composite populations (1.39 g d^{-1} for WR97, 1.62 g d^{-1} for WR99, and 1.82 g d^{-1} for WR00). These responses increased by 0.215 ± 0.003 ($P < 0.01$) with each incremental increase in selection pressure from WR97 to WR99 to WR00. These three populations did not differ in linear responses of root mass to post-emergence time.

As early as 16 d post-emergence, a large amount of additive genetic variation was observed for seedling shoot and root fresh mass in reed canarygrass, as evidenced by correlated selection responses. A large portion of this variation appears to be related to the improved establishment capacity of the selected populations. Three cycles of selection for seedling vigor in sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.] increased seedling height by 8% cycle^{-1} and stand density by 13% cycle^{-1} (Voigt and Brown, 1969). Two cycles of selection for seedling height in alfalfa increased field emergence by 4% cycle^{-1} at one of two locations (Klos and Brummer, 2000b). Thus, seedling vigor appears to be a key component to seedling establishment across a wide array of perennial species. Contrasting results have been observed for switchgrass, in which selection for increased seedling mass resulted in no significant changes in either shoot or root mass measured in the field (Smart et al., 2003).

Mean date of tiller initiation responded inconsistently to selection, decreasing by 3.6 and 6.8% in Vantage and Venture, respectively, but increasing by 5.0% in Rival (Table 7). Tiller number 42 d post-emergence decreased

Table 7. Mean days to tiller initiation and number of tillers 40 d after emergence for seven reed canarygrass cultivars or populations and their progeny selected for establishment capacity. Means are over 12 replicates and two runs (Experiment 4c).

Cultivar or population	Tiller initiation			Tiller number		
	Original	Selected	*P* value	Original	Selected	*P* value
	d			no.		
Bellevue	20.9	21.2	0.57	5.0	4.7	0.02
Palaton	21.9	21.8	0.79	4.3	4.5	0.22
Rival	20.7	21.7	0.01	4.7	4.6	0.34
Vantage	22.2	21.4	0.04	4.4	4.5	0.68
Venture	23.1	21.5	<0.01	4.8	4.3	<0.01
WR99	22.4	21.9	0.22	4.2	3.3	0.32
WR00	22.4	21.7	0.07	4.2	4.3	0.45
LSD(0.05)	0.7			0.3		

by 6.6 and 11.4% in Bellevue and Venture, respectively, as well as averaged across the five cultivars (3.3%). Neither mean date of tiller initiation nor tiller number responded to selection for the composite populations. In switchgrass, divergent selection for seedling tiller number had no effect on seedling establishment capacity (Smart et al., 2003). Tillering per se was not a factor regulating genetic variability for establishment capacity in our study or the switchgrass study.

Synthesis and Conclusion

Seedling vigor is generally believed to be the single most important variable regulating genetic variability in establishment capacity of perennial forage crops. Many researchers have used some measure of seedling vigor as a selection criterion to improve establishment capacity, while others have used more indirect measures, such as seed mass or germination rate. Nevertheless, those in the latter group have generally demonstrated correlated improvements in seedling vigor as a result of selection. Similarly, across the 13 reed canarygrass populations evaluated in this study, seedling fresh mass at 30 d post-emergence (SM30) was the most important variable regulating establishment capacity. Multiple regression, using backward stepwise elimination ($P < 0.01$), eliminated all other variables from the regressions for ground cover (GC) and tiller density (TD): GC = $-0.272 + 0.778$(SM30), $R^2 = 0.80$; TD = $-6.07 + 3.23$(SM30), $R^2 = 0.75$.

Our study was unique among attempts to improve establishment capacity in that selection was based on the most direct measure of establishment available to us—survival, sexual reproduction, and fecundity of 1-yr-old plants. Harvesting seed from all families and retaining each family utilized 3/4 of the additive genetic variance within the population as a whole (Falconer, 1989). Furthermore, plants were allowed to reproduce and contribute gametes to the next generation in direct proportion to their size or number of flowering tillers, potentially increasing selection pressure for establishment capacity, as dictated by 1-yr-old plant size. Because selection was based directly on establishment capacity, there was no need to rely on strong genetic correlations between establishment capacity and an alternative selection criterion, such as seedling vigor. The observation of genetic gains within all five cultivars and the pooled populations indicated a certain level of repeatability of this selection protocol across populations.

Despite the overall importance of seedling fresh mass at 30 d post-emergence, there was considerable variability among cultivars in the nature of their correlated responses. For Palaton, WR99, and WR00, increased shoot mass and root mass indicated that improved seedling vigor was the mechanism for improved establishment capacity. For Vantage and Venture, increased shoot mass and root mass, combined with faster tiller initiation, suggested that both seedling vigor and tillering rate were responsible for improved establishment capacity. For Bellevue and Rival, there were no significant changes for any seedling trait, suggesting an unknown mechanism was responsible for improved establishment capacity. The variability among these responses and the implication of multiple mechanisms for improving establishment capacity is further evidence that direct selection for some measure of establishment capacity is probably the most efficient selection method to improve this trait of reed canarygrass.

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